International Journal of Pharmaceutics 528 (2017) 76–87

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Comparison of different zeolite framework types as carriers for the oral delivery of the poorly soluble drug indomethacin

Christina Karavasili[a], Elsa P. Amanatiadou[b,c], Eleni Kontogiannidou[a], Georgios K. Eleftheriadis[a], Nikolaos Bouropoulos[d,e], Eleni Pavlidou[f], Ioanna Kontopoulou[d], Ioannis S. Vizirianakis[b], Dimitrios G. Fatouros[a,*]

[a] Laboratory of Pharmaceutical Technology, School of Pharmacy, Aristotle University of Thessaloniki GR, 54124, Greece
[b] Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki GR, 54124, Greece
[c] Saint Paul General Hospital, GR-55134 Thessaloniki, Greece
[d] Department of Materials Science, University of Patras, 26504 Rio, Patras, Greece
[e] Foundation for Research and Technology, Hellas-Institute of Chemical Engineering Sciences – FORTH/ICE-HT, P.O. Box 1414, GR-26504 Patras, Greece
[f] Department of Physics, Aristotle University of Thessaloniki GR, 54124, Greece

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 24 May 2017
Accepted 25 May 2017
Available online 30 May 2017

Keywords:
Zeolites
Poorly soluble drugs
Oral delivery
Cytocompatibility
Simulated intestinal fluids

ABSTRACT

Microporous zeolites of distinct framework types, textural properties and crystal morphologies namely BEA, ZSM and NaX, have been employed as carriers to assess their effect on modulating the dissolution behavior of a BCS II model drug (indomethacin). Preparation of the loaded carriers *via* the incipient wetness method induced significant drug amorphization for the BEA and NaX samples, as well as high drug payloads. The stability of the amorphous drug content was retained after stressing test evaluation of the porous carriers. The dissolution profile of loaded indomethacin was evaluated in simulated gastric fluid (pH 1.2) and simulated intestinal fluids FaSSIF (fasted) and FeSSIF (fed state) conditions and was found to be dependent on the aluminosilicate ratio of the zeolites and the degree of crystalline drug content. The feasibility of the zeolitic particles as oral drug delivery systems was appraised with cytocompatibility and cellular toxicity studies in Caco-2 cultures in a time- and dose-dependent manner by means of the MTT assay and flow cytometry analysis, respectively. Intracellular accumulation of the zeolite particles was observed with no apparent cytotoxic effects at the lower concentrations tested, rendering such microporous zeolites pertinent candidates in oral drug delivery applications.

1. Introduction

Limited drug solubility in aqueous media constitutes a primal restraint in the formulation development of orally administered dosage forms, since it is the rate limiting factor to the concomitant absorption and bioavailability processes (Fahr and Liu, 2007). The intervention in the molecular mobility of a drug candidate is among the approaches commonly pursued to effectively confront solubility limitations. This approach accounts for the conversion of the thermodynamically stable crystalline form of a drug compound into its amorphous state of higher free energy and increased solubility and the long-term stabilization of the amorphous form to prevent unwilling recrystallization upon storage. For the drugs categorized as BCS class II, that exhibit low solubility but reasonable membrane permeability, the rate-limiting process of absorption is the drug dissolution step. Therefore, formulation is a key factor in determining the rate and extent of drug absorption from the gastrointestinal tract (Pouton, 2006).

During the past decades a diversity of porous materials has been developed, inducing a significant impetus for a vast range of potential applications. Apart from their extensive industrial uses, their intriguing properties, including their large surface area, uniform pore size and adjustable composition and structure, have earned porous materials remarkable applicability in biomedical applications, as well (Arruebo, 2012). Most importantly, they have been reported to be non-toxic and biocompatible, rendering them suitable for utilization in pharmaceutical formulations (Elmore, 2003). In particular, mesoporous silica nanoparticles, (Mellaerts et al., 2008; Shen et al., 2010; Vallet-Regí et al., 2001) silicon nanoparticles (Salonen et al., 2005), metal organic frameworks (McKinlay et al., 2010; Horcajada et al., 2010) and mesoporous carbons (Karavasili et al., 2013; Gencoglu et al., 2014; Eleftheriadis

* Corresponding author.
E-mail address: dfatouro@pharm.auth.gr (D.G. Fatouros).

http://dx.doi.org/10.1016/j.ijpharm.2017.05.061
0378-5173/

et al., 2016) have been widely appraised as prospective carriers for drug delivery and diagnostic applications. Recently, zeolites have also attracted substantial attention as carriers for the delivery of anti-neoplastic compounds (Datt et al., 2013; Spanakis et al., 2014; Grund et al., 2012), DNA and nucleic acids (Lülf et al., 2014; Bertucci et al., 2014) and both hydrophilic (Fatouros et al., 2011; Paradee and Sirivat, 2016) and lipophilic drugs (Datt et al., 2012; Karavasili et al., 2016). Zeolites constitute a group of crystalline inorganic aluminosilicates with uniform microporous three-dimensional architecture and enormous internal surface areas of values even close to 1000 m^2/g, accounting for their characterization as molecular sieve adsorbents. In that context, they have been deployed as pertinent pharmaceutical carrier systems to improve the dissolution of drugs with an intrinsic low aqueous solubility and effectively modulate drug release in a predictable and reproducible manner (Danilczuk et al., 2008). The utilization of mesoporous materials and hierarchical zeolites has been previously defined as a convenient approach to regulate drug release rates (García-Muñoz et al., 2014; Andersson et al., 2004). Nevertheless, there are few studies delineating the influence of zeolites' structural and textural properties on the drug loading and release performance of the hierarchical materials (Datt et al., 2013, 2012; Fatouros et al., 2011).

In the current work, we report on the feasibility of three different zeolite framework types as hosts of the BCS II model compound indomethacin, with the aim to modulate the dissolution behavior of the poorly water-soluble drug intended for oral administration, based on the individual zeolite microstructure. To the best of our knowledge this is the first report recording the release profiles of APIs from zeolites in simulated intestinal fluids coupled with toxicity studies in Caco-2 monolayers, a human intestinal cell line, by means of MTT and FACS analysis, trans-epithelial electrical resistance (TEER) measurements, an *in vitro* probe to assess the membrane integrity and uptake studies by fluorescence microscopy.

2. Materials and methods

Indomethacin, molecular sieves (13 × powder, ~2 μm average particle size) NaX-FaU (Si/Al: 1.2), acetonitrile and water for HPLC were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were of analytical grade. Distilled water was used in all experimental procedures. CP814E (Si/Al: 25) [BEA] and CBV2314 (Si/Al: 23) [ZSM-5] were obtained from Zeolyst (US).

2.1. Cell culture

The Caco-2 human colorectal adenocarcinoma cell line was cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin and 0.1% non-essential amino acids. Cells were grown at 37 °C in a humidified chamber of an incubator with 5% CO_2 v/v. Caco-2 cells were used at a passage number between 40 and 45.

2.2. Preparation of drug loaded zeolite carriers

Incipient wetness impregnation (I.W.) was the method employed for the drug loading of zeolites. BEA, ZSM and NaX zeolites were overnight oven dried at 120 °C. Zeolitic powders were then gradually impregnated with an ethanolic solution (T = 75 °C) of indomethacin (zeolite to drug weight ratio 2:1) and vigorously ground with a pestle, till apparent dryness. Loaded samples were then dried at 40 °C under vacuum for 48 h to remove any residual organic solvent. Zeolitic carriers were stored in a desiccator under $CaCl_2$ till further analysis.

2.3. Drug content quantification

Thermogravimetric analysis (TGA) was carried out using a TGA Q500 (TA instruments Ltd.) apparatus with a heating rate of 10 °C/min from 40 °C to 800 °C in air atmosphere. Prior measurements, samples were equilibrated at 40 °C to enable removal of the excess moisture content. The drug content was calculated according to the Eq. (1), after appropriate corrections accounting for the weight loss of empty zeolites in the same temperature ranges. Drug content was additionally quantified *via* an 'extraction' method under sink conditions. One (1) mg of each loaded zeolite was suspended in 10 mL PBS solution in the presence of 2% w/v sodium lauryl sulfate (SLS) and stirred overnight. Dispersions were then centrifuged at 4500 rpm for 30 min and supernatants were filtered through 0.2 μm syringe filters (Whatman Inc.), prior UV quantification (UV mini-1240, SHIMADZU) at 230 nm.

$$\text{drug content}(\%) = \frac{\text{weight of indomethacin in the zeolites}}{\text{weight of zeolites}}(x)100 \quad (1)$$

2.4. Physicochemical characterization

2.4.1. Scanning electron microscopy (SEM)

Zeolites were visualized by means of scanning electron microscopy (SEM) analysis. Scanning electron microscopy (SEM) was carried out using a JEOL JSM-6390LV scanning microscope equipped with an energy – dispersive X-ray (EDS) INCA micro-analytical system. Operating conditions were: accelerating voltage 20 kV, probe current 45 nA and counting time 60 s, with ZAF correction being provided on-line. The samples were coated with carbon using a Jeol JEE-4X vacuum evaporator.

2.4.2. ζ-potential measurements

ζ-potential measurements were performed on a Zetasizer Nanoseries, Nano-ZS analyzer (Malvern, UK) at 25 °C. Empty and drug loaded zeolitic carriers were dispersed in distilled water at a final concentration of 1 wt.% and sonicated prior to analysis. Measurements were repeated in triplicate.

2.4.3. Particle size distribution

The particle size distribution of the different zeolite types was assessed with a Mastersizer 2000 (Malvern UK) laser diffraction equipped with a Hydro 2000G (Malvern) wet dispersion sampler. The samples were dispersed in d. water and the pump and stirrer speeds were adjusted at 2000 rpm and 1000 rpm, accordingly.

2.4.4. Physisorption studies

N_2 adsorption/desorption isotherms were determined using a Nova 2200E Surface Area and Pore Size Analyzer (Quantachrome Instruments, Boynton Beach, Florida) at −196 °C to maintain isothermal conditions. All samples were degassed at 50 °C for 24 h prior to analysis. The specific surface areas were calculated at relative pressures $P/P_o = 0.02–0.04$ using the Brunauer-Emmett-Teller (BET) method, while micropore areas and external surface areas were deduced by the t-plot method at relative pressures $P/P_o = 0.2–0.4$. Pore size distributions were determined *via* the Saito-Foley (SF) approximation for cylindrical pore geometry. Total pore volumes were estimated from the amount of N_2 adsorbed at a relative pressure of 0.995.

2.4.5. Differential scanning calorimetry (DSC)

DSC analysis was conducted on a DSC 204 F1 Phoenix (Netzsch) apparatus at a heating rate of 10 °C/min under a nitrogen purge of